Applied and Environmental Microbiology, Dec. 2011, p. 8595–8604 Vol. 77, No. 24
0099-2240/11/$12.00 doi:10.1128/AEM.06175-11

Butyric Acid- and Dimethyl Disulfide-Assimilating Microorganisms in a Biofilter Treating Air Emissions from a Livestock Facility▽

Anja Kristiansen,[1] Sabine Lindholst,[2] Anders Feilberg,[3] Per H. Nielsen,[1] Josh D. Neufeld,[4] and Jeppe L. Nielsen[1]*

Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Sohngaardsholmsvej 49, DK-9000 Aalborg, Denmark[1]; Department of Chemistry and Biotechnology, Danish Technological Institute, Kongsvang Allé 29, DK-8000 Aarhus C, Denmark[2]; Department of Agricultural Engineering, Aarhus University, Blichers Allé 20, DK-8830 Tjele, Denmark[3]; and Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, Canada[4]

Received 13 July 2011/Accepted 6 October 2011

Biofiltration has proven an efficient tool for the elimination of volatile organic compounds (VOCs) and ammonia from livestock facilities, thereby reducing nuisance odors and ammonia emissions to the local environment. The active microbial communities comprising these filter biofilms have not been well characterized. In this study, a trickle biofilter treating air from a pig facility was investigated and proved efficient in removing carboxylic acids (>70% reduction), mainly attributed to the primary filter section within which reduced organic sulfur compounds were also depleted (up to 50%). The secondary filter eliminated several aromatic compounds: phenol (81%), *p*-cresol (89%), 4-ethylphenol (68%), indole (48%), and skatole (69%). The active butyric acid degrading bacterial community of an air filter sample was identified by DNA stable-isotope probing (DNA-SIP) and microautoradiography, combined with fluorescence *in situ* hybridization (MAR-FISH). The predominant 16S rRNA gene sequences from a clone library derived from "heavy" DNA from [$^{13}C_4$]butyric acid incubations were *Microbacterium*, *Gordonia*, *Dietzia*, *Rhodococcus*, *Propionibacterium*, and *Janibacter*, all from the *Actinobacteria*. *Actinobacteria* were confirmed and quantified by MAR-FISH as being the major bacterial phylum assimilating butyric acid along with several *Burkholderiales*-related *Betaproteobacteria*. The active bacterial community assimilating dimethyl disulfide (DMDS) was characterized by DNA-SIP and MAR-FISH and found to be associated with the *Actinobacteria*, along with a few representatives of *Flavobacteria* and *Sphingobacteria*. Interestingly, ammonia-oxidizing *Betaproteobacteria* were also implicated in DMDS degradation, as were fungi. Thus, multiple isotope-based methods provided complementary data, enabling high-resolution identification and quantitative assessments of odor-eliminating *Actinobacteria*-dominated populations of these biofilter environments.

The increased implementation and productivity of large-scale pig farms have increased nuisance air emissions of both ammonia and volatile organic compounds (VOCs). Deposition of ammonia emissions in environmental recipients can result in eutrophication, with devastating consequences for the fauna present (47). Odor nuisance of the more than 300 different VOCs originating from pig facilities has been reported at concentrations irritating humans, individually or through synergistic reactions (43). Thus, reducing air emissions of ammonia and odorous VOCs from pig facilities is important for the interests of farming, environmental and public sectors.

Biological airfilters have proven useful in reducing a wide range of compounds emitted from pig production facilities (11). Biofilters rely on the versatile metabolic pathways of microorganisms, which are exposed to air contamination and transform these compounds into biomass, carbon dioxide, and water, as well as waste products. These waste products are washed away by "trickle" action, thereby maintaining the biofilter in continuous adsorptive capacity (8). The main advantages of biofilters compared to physical and chemical remediation are (i) the ability to remove low concentrations of pollutants from large air volumes, (ii) limited maintenance, and (iii) low operation costs (40).

Butyrate is a predominant odor compound emitted from pig facilities. Through acidogenic fermentation, butyrate is produced at concentrations up to 60 ppb. With a human detection threshold of around 4 ppb, butyrate is recognized as a sour fecal stench (43). Commonly detected sulfur compounds include methanethiol (odor threshold value, ~1 ppb), dimethyl sulfide (odor threshold value, ~2 ppb), and dimethyl disulfide (DMDS; odor threshold value, ~12 ppb), and these represent intermediates of the methionine and cysteine degradation (43). Full-scale biofiltration of emissions from a pig facility revealed stable removal of carboxylic acids in the range of 70 to 90%, whereas the reduction of reduced organic sulfur compounds fluctuated between −20 to 50% (11). Removal efficiencies of DMDS ca. 100% have, however, been obtained in a full-scale biofilter treating air emissions from a composting facility (17).

Although the physical and chemical factors affecting biofilters are fairly well examined (e.g., support material, pH, and moisture conditions), only limited information is available on the biological aspects (8, 44). Few molecular studies have provided descriptions of the microbial communities associated with biofilters treating air emissions (14, 15, 20, 52). Compre-

* Corresponding author. Mailing address: Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Sohngaardsholmsvej 49, DK-9000 Aalborg, Denmark. Phone: 459 9408-506. Fax: 459 8141-808. E-mail: jln@bio.aau.dk.

▽ Published ahead of print on 14 October 2011.

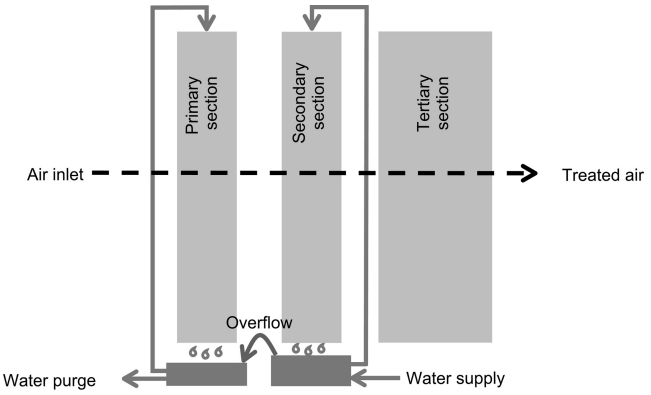

FIG. 1. Schematic diagram of biofilter in Kiel (Germany). Airflow (broken arrow) and water flow (solid arrows) in the primary filter and secondary filter section is indicated. The only modification with respect to the biofilter investigated in Randers (Denmark) was the absence of the tertiary filter. (Modified with permission from SKOV A/S.)

hensive characterization and understanding of air biofilter metabolism requires knowledge about *in situ* metabolic function and activity. The use of stable-isotope probing (SIP) in combination with phospholipid fatty acid analysis on lab-scale biofilter samples identified *Pseudomonas* as a part of the styrene-degrading community (1) and *Thiobacillus* as an active member of the autotrophic community in a dimethyl disulfide-treating biofilter (25). Findings by Friedrich and Lipski (13), who also combined isotope labeling and PLFA to a full-scale oil mill biofilter, revealed a central role of *Gordonia* in hexane transformations. However, no information from molecular studies is available on the metabolic role of microorganisms in full-scale biofilters treating air from pig facilities, and no study has yet used DNA-based stable-isotope probing (DNA-SIP) to characterize active biofilter biofilm populations.

Although the ability to remove a large range of different odor compounds emitted from pig facilities using biofiltration is increasingly used, the microbiology in these filters remains almost unknown (8). The aim of this study was to identify microorganisms involved in the transformation of butyric acid and DMDS in a full-scale biological air filter treating air from a pig facility. The air-water partitioning of DMDS is in the same order of magnitude as other more abundant sulfur compounds (e.g., methanethiol and dimethyl sulfide). Application of DNA-SIP allowed us to identify active bacteria involved in degradation of specific odor compounds in a full-scale biological air filter treating exhaust air from a pig farm; the results were confirmed and quantified using microautoradiography combined with fluorescence *in situ* hybridization (MAR-FISH).

MATERIALS AND METHODS

Biofilter description and analysis. From geographically distinct finishing facilities, two similar full-scale biofilters of the SKOV-type (SKOV, Roslev, Denmark) were examined in the present study (Fig. 1). One was located near Randers (Denmark) and used to measure the reduction efficiency of selected compounds and another was located near Kiel (Germany) and used for both substrate reduction kinetics and microbial analyses. The two-sectioned biofilter in Randers was of the trickling type and composed of porous corrugated cellulose pads (each 2 by 10 by 0.3 m). The filter was irrigated with water to support the active biofilm and wash away toxic waste productions. The total empty bed residence time for this type of filter is around 0.3 to 0.4 s, and the pressure drop in the order of 12 to 20 Pa. The Kiel biofilter was identical to the Randers filter except of the dimensions (2 by 1.8 by 0.15 m) and an additional nonhumidified filter section (2 by 1.8 by 0.3 m) located in continuation to the first two sections (Fig. 1) in order to remove reminiscent hydrophobic compounds.

Biofilter performance. The pig stable-air emission and removal efficiencies of each biofilter compartment were analyzed by using thermal desorption gas chromatography-mass spectrometry (TD-GC/MS) and membrane inlet mass spectrometry (MIMS; QMG 422; Balzers, Liechtenstein) according to a previously developed method (11). Briefly, insulated polyterafluoroethylene tubes of equal length were installed in (i) the ventilation channel before the biofilter, (ii) between the two filter sections, and (iii) after the filter used for air sampling. The quadrupole instrument was equipped with an axial electron impact ion source (70 eV) and a mass-to-charge (*m/z*) range of 0 to 200. The custom-built membrane inlet (Mikrolab, Denmark) was holding a poly-(dimethylsiloxane) sheet membrane (130 μm thickness; Technical Products, Lawrenceville, GA). The inlet temperature was held at 50°C, and the sample flow over the membrane was 300 ml min^{-1}. Online measurements of selected *m/z* values were conducted: *m/z* 60 (carboxylic acids), *m/z* 94 (phenol), *m/z* 108 (*p*-cresol), *m/z* 122 (4-ethylphenol), *m/z* 117 (indol), *m/z* 130 (skatole), and *m/z* 47 (reduced organic sulfur compounds).

Measurement of dimethyl disulfide in the Randers filter was conducted together with the analysis of methanethiol, dimethyl sulfide, and H_2S by proton transfer reaction mass spectrometry (PTR-MS) analysis at a single time point (11). Briefly, 5 liters of air were collected in nalophan bags and transported directly to the laboratory. Concentrations were measured on a high-sensitivity PTR-MS (Ionicon, Austria) by detection of *m/z* 35 (H_2S), *m/z* 49 (methanethiol), *m/z* 63 (dimethyl sulfide), *m/z* 95 (DMDS and phenol), and *m/z* 97 (^{34}S-labeled isotope of DMDS). In order to correct *m/z* 95 for a contribution from phenol, additional samples were collected on Tenax TA adsorption tubes (Markes, United Kingdom) and analyzed for phenol by gas-chromatography with mass spectrometry (GC/MS; Agilent) as described elsewhere (11), with the exception that pure Tenax TA was used as an adsorbent. Sample introduction was obtained by thermal desorption (Perkin-Elmer Turbomatrix).

Samples of air for analysis by GC/MS were collected by a method comparable to the method recommended by Trabue et al. (50) for sampling in humid environments. Air was drawn through a sorbent tube packed with Tenax TA and Carbograph 5D (Markes International, United Kingdom) at a rate of 100 ml/min for 15 min. The tubes were sealed and subsequently analyzed for odorous compounds in the laboratory using thermal desorption coupled with GC/MS. Thermal desorption (Turbomatrix ATD400; Perkin-Elmer) was carried out as follows: primary desorption at 300°C, cryogenic focusing on Tenax TA at −30°C, and secondary desorption at 300°C min. An Agilent GC/MS (Agilent 6890N GC and 5973N MS) was used for chromatographic separation using a temperature programmed DB5-MS column (30 m; inner diameter, 0.25 mm; 0.25-μm film thickness; J&W Scientific) and for compound detection and identification by full-scan MS. Calibration was performed by injection of known amounts of odorants dissolved in pentane or methanol onto sorbent tubes. A significant fraction of methanethiol will be converted to dimethyl disulfide during sampling and analysis (11). The measured dimethyl disulfide concentration is therefore due to a combination of converted methanethiol and dimethyl disulfide originally present in sampling air.

Emission and removal efficiency of ammonia was estimated using the Matheson-Kitagawa toxic gas detector system (MEGS Specialty Gas) at a single time point (the detection limit was ~0.5 ppm).

Physical-chemical analyses. The pH and the concentration of nitrate and nitrite in the irrigation water were determined according to the European standards EN ISO 10523:2008 and EN ISO 13395:1996, respectively. The pH in both the primary and the secondary filter was 7 for the Randers filter, whereas 7.1 and 7.2, respectively, were used for the Kiel biofilter. In the irrigation water of the Randers biofilter, inorganic N was extracted in a 1 M KCl solution as described by Keeney and Nelson (22), and nitrate and nitrite concentrations were determined colorimetrically using an autoanalyzer (Technicon TRAACS 800; Bran+Luebbe GmbH, Norderstedt, Germany). In the Randers biofilter, the nitrate concentrations were found to be 131 ± 11 mM and 90 ± 8 mM, while the nitrite concentrations were 2.1 ± 1 mM and 7.0 ± 2 mM in the primary and secondary filters ($n = 6$), respectively. In the Kiel biofilter nitrate was below 0.7 mM in both filters, and the nitrite concentrations were 0.13 ± 0.1 mM and 0.04 ± 0.03 mM ($n = 6$), respectively.

Sample collection and microbial isotope labeling. Representative biofilter biofilm samples ($n = 6$) from the Kiel facility were scraped off the support material across the first filter, kept cool (4°C), and transported directly to the laboratory for subsequent analysis. The biofilm were suspended in 0.01 mM

phosphate buffer (pH 7.2) with 200 μM thiourea added to maintain pH and inhibit nitrification (to a final concentration of 10 g liter^{-1}). This same solution was used for three different incubations in the present study: (i) DNA-SIP analysis, (ii) MAR-FISH analysis, and (iii) substrate consumption rate measurements. Multiple substrate doses were added to SIP incubations in order to keep the concentrations of butyric acid and DMDS low and as close to *in situ* conditions as possible while still incorporating detectable amounts of ^{13}C into the active biomass.

(i) SIP incubations. A total of 9 ml of the biofilm solution was transferred to 60-ml serum bottles, and either 3 mM [$^{13}C_4$]butyric acid (Isotec, Miamisburg, OH) or 0.01 mM [$^{13}C_2$]DMDS (Isotec) was added as a substrate. Parallel incubations were also prepared with unlabeled substrate and used as controls for verification of DNA-SIP labeling. The bottles were crimp sealed with rubber stoppers and incubated at 20°C on a rotary table. When the headspace substrate concentration approached 0 ppb, 3 mM butyric acid (labeled or unlabeled) was again added to bottles incubated with butyric acid as a substrate. Butyric acid was added to time course incubations a total of 1, 5, or 13 times for samples incubated for 7, 45, and 110 h, respectively. Similarly, 0.01 mM DMDS (labeled or unlabeled) was fed to bottles incubated with DMDS as a substrate either one or two times, giving total incubation times of 120 and 240 h, respectively. For each time point, incubation samples were frozen at −80°C until DNA-SIP analysis.

(ii) MAR-FISH incubations. MAR-FISH was performed as described previously (38). Briefly, 2 ml of the biofilm solution was transferred to 9-ml serum bottles, and either 20 μCi of [$^{14}C_2$]butyric acid (3 mM; American Radiolabeled Chemicals, St. Louis, MO) or 10 μCi of [$^{14}C_2$]DMDS (0.1 mM; American Radiolabeled Chemicals) was added. Bottles were crimp sealed with rubber stoppers and incubated aerobically at 20°C on a rotary table. As a control for chemography, a biofilm solution was pasteurized at 70°C for 10 min prior to MAR incubation. Incubation times were 19 and 260 h, respectively, for butyric acid and DMDS as a substrate. MAR incubations were carried out in triplicates and terminated by fixing samples with 4% paraformaldehyde and 50% ethanol for Gram-negative and Gram-positive bacteria, respectively, as described elsewhere (37).

(iii) Consumption rate measurements. Triplicate 2-liter reference bottles with 0.25 liter of the biofilm solution and 3 mM nonlabeled butyric acid (Sigma-Aldrich, St. Louis, MO) or 0.01 mM nonlabeled DMDS (Sigma-Aldrich) were incubated under the same conditions as described for SIP and MAR incubations. The gas-tight lids were penetrated by polytetrafluoroethylene tubes for headspace measurements of butyrate (*m*/*z* 60) and DMDS (*m*/*z* 94 and *m*/*z* 96) by MIMS using settings as described previously. For MIMS, the lowest possible airflow of 20 ml min^{-1} and recirculation of the headspace air sample was applied in order to reduce the loss of butyrate and DMDS during sampling. In bottles identical to the reference setups but with no biofilm added, loss of substrates during MIMS measurement was determined and found to be insignificant. To estimate the vapor-liquid equilibrium of butyric acid and DMDS, Henry's constants of 1.90×10^3 M atm^{-1} (5) and 8.40×10^{-1} M atm^{-1} (51) for standard conditions were used, although they were likely deviating from authentic values due to ions and biofilm in the liquid phase and a 5°C temperature difference.

Samples for pH detection were taken from the reference bottle using a needle and syringe and measured by indicator strips (Merck). The pH of the butyric acid incubation was adjusted periodically by titration with 1 M NaOH; an equal concentration was added to the SIP and MAR incubations.

DNA extraction and fractionation. Total DNA was extracted from the SIP incubations after 7, 45, and 110 h of incubation for [$^{13}C_4$]butyric acid and for 120 and 240 h of incubation for [$^{13}C_2$]DMDS, and 2-liter reference bottles using the Fast DNA SPIN kit (MP Biomedicals, Irvine, CA). The DNA concentration was measured on a NanoDrop 2000 spectrophotometer (Thermo Scientific). Isopycnic separation of "heavy" and "light" DNA was performed on each DNA extract as described previously (36). Briefly, from each sample between 0.24 and 2 μg of DNA was added to the gradient buffer and mixed with cesium chloride to a final density of 1.725 g ml^{-1}. Solutions were sealed in 5.1-ml polyallomer tubes (Beckman Coulter, Fullerton, CA) and ultracentrifuged in a Vti 65.2 vertical rotor (Beckman Coulter) at 20°C with a speed of 177,000 × g_{av} for 40 h. Each tube was fractionated into 12 volumes of ~425 μl, and DNA was precipitated with polyethylene glycol and glycogen (Sigma-Aldrich), which was verified by PCR to be a nucleic-acid-free carrier for precipitation (3). Fractionated DNA was suspended in nuclease-free water, and aliquots were visualized on a 1% agarose gel stained with GelRed (Biotium, Hayward, CA).

Fingerprinting of fractionated DNA. PCR amplification for denaturing gradient gel electrophoresis (DGGE) analysis was performed using the bacterial primer pair 341F-GC and 518R (34). Amplification was performed in a volume of 50 μl using the following conditions: 1× ThermoPol Buffer (New England Biolabs, Ipswich, MA), 0.03 mg of bovine serum albumin (Sigma-Aldrich), 0.4 mM deoxynucleoside triphosphate, 0.5 μM concentrations of each primer, and 2.5 U of *Taq* polymerase (New England Biolabs). A C1000 thermal cycler (Bio-Rad) was used for the amplification starting with 5 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C, and these were followed by a final extension step of 7 min at 72°C. PCR products were run on a 10% acrylamide gel with 30 to 70% denaturant as described previously (16); electrophoresis was carried out at 85 V and 60°C for 14 h with a DGGEK-2401 system (CBS Scientific). The gels were stained for 1 h with SYBR green (Invitrogen) and scanned with a Typhoon 9400 variable mode imager (GC Healthcare).

16S rRNA gene clone library construction and phylogenetic analysis. From the DGGE results, distinct light (fraction 10) and heavy (fraction 5) DNA fractions were identified. Predominant bands from fraction 5 were chosen for subsequent sequencing by excising bands, reamplification using the protocol described above, and sequencing with the 341F primer.

PCR products were generated using the 16S rRNA gene primer pair 8F and 1492R (12, 27), targeting most bacteria. The 50-μl PCR mix contained 1× standard reaction buffer (Amplicon, Skovlunde, Denmark), 0.5 mM deoxynucleoside triphosphate (Amplicon), 0.2 μM concentrations of each primer, 2.5 U of *Taq* DNA polymerase (Amplicon), and 1 μl of DNA template. The PCR amplification was initiated by 10 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 56°C, and 30 s at 72°C, and these steps were followed by a final extension step of 7 min at 72°C. Amplicons of the 16S rRNA gene were ligated directly into the TOPO vector (Invitrogen) and transformed as described in the manufacturer's instructions. Recombinant plasmids were extracted using the FastPlasmid minikit (Eppendorf, Lübeck, Germany) and sequenced by Macrogen (Korea).

The Bellerophon server (18) identified eight putative chimeric sequences, and these were excluded from further analysis. Sequences were aligned and phylogenetic trees were constructed in the ARB software package (30) with the SILVA reference database (release 100 [39]) using maximum-likelihood, neighbor-joining, and maximum-parsimony algorithms. Only near-full-length sequences (>1,200 nucleotides) were included, and a filter based on at least 50% base similarity was applied. Bootstrap analysis of the branching pattern was made with the maximum-parsimony method (1,000 replicates). Clones obtained in the present study and represented in the phylogenetic trees were named butyric acid-oxidizing biofilm (BOB) clones. The sequences obtained here are available in GenBank under accession numbers HQ116699 to HQ116760.

FISH and microautoradiography. Samples were homogenized in a glass tissue grinder (Thomas Scientific) and immobilized on gelatin-coated coverslips at 50°C. The samples were hybridized with fluorescently labeled oligonucleotide probes (Biomers, Ulm, Germany) performed as described in detail elsewhere (37). The EUBmix probes (9, 49) were labeled with 5(6)-carboxyfluorescein-*N*-hydroxy-succinimide ester (FLUOS). Phylum- and group-specific probes were labeled with sulfoindocyanine dyes (Cy3), as was the NONEUB negative control probe (53). Phylum-specific probes used in the present study included BONE23a (2), BTWO23a (2), NSO190 (33), BET42a, GAM42a (32), CF319ab (31), and HGC69a (42), and these corresponded to the β1 group of *Betaproteobacteria*, the β2 group of *Betaproteobacteria*, *Nitrosomonas*, *Gammaproteobacteria*, *Bacteroidetes*, and *Actinobacteria* groups, respectively. When possible, a hierarchical set of probes was used to increase probe specificities. Quantification of fluorescence *in situ* hybridization (FISH) signals was performed as described by Nielsen (37) as a double hybridization with Cy3-labeled specific probes and the FLUOS-labeled probe EUBmix. The detection limit was 0.3% of EUBmix-positive cells as determined with the Cy3-labeled probe NONEUB. The FISH-detectable fraction of the community was determined relative to the total prokaryotic community, that is, cells with a diameter <2 μm as stained with DAPI (4′,6′-diamidino-2-phenylindole; 1 μg ml^{-1}; Molecular Probes) after FISH for 10 min. Autoradiographic processing and microscopic evaluations were conducted as described by Nielsen and Nielsen (38).

RESULTS

Biofilter performance. The two biofilters (Randers and Kiel) had been running constantly for several months at the time of sampling. The cellulose pad biofilm exhibited a patchy distribution of various thicknesses. There was an overall decrease in biofilm thickness along the airflow axis from several millimeters to barely discernible. The support material of the third section of the Kiel biofilter was covered by a fibrous layer of fungal mycelium-like morphology. The pH in both biofilters

TABLE 1. Concentrations of selected VOCs and their removal efficiency in the Kiel and Randers trickling biofilters

Analysis type	*m/z* value	Concn (ppb)		Removal efficiency (%)					
				Kiel filter			Randers filter		
		Kiel	Randers	Overall	Primary section	Secondary section	Overall	Primary section	Secondary section
Measurement by Matheson-Kitagawa gas detection									
Ammonia		~8,000	~10,000	89 ± 10			90 ± 10		
Organic compounds[a]									
Acetic acid	60	910 ± 20.3		~100	~100				
Propionic acid	74	330 ± 4.3		99.8	99.7	29.8			
2-Methylpropionic acid	88	33.6 ± 1.4		~100	~100				
Butyric acid	88	116 ± 2.8		99.9	99.9				
3-Methylbutyric acid	102	20.4 ± 0.3		~100	~100				
2,3 Butanedione	86	10.2 ± 4.5		~100	~100				
3-Hydroxy-2-butanone	88	28.3 ± 22.3		~100	~100				
Pentanoic acid	102	27.7 ± 1.2		~100	~100				
4-Methylpentanoic acid	132	0.12 ± 0.2		~100	~100				
Hexanoic acid	116	3.2 ± 0.6		~100	~100				
$C_{>2}$ carboxylic acids	73			~100	~100		70 ± 18	53 ± 25	42 ± 20
Phenol	94	3.8 ± 0.6		~100	~100		81 ± 3	44 ± 9	66 ± 6
p-Cresol	108			~100	~100		89 ± 3	46 ± 10	79 ± 4
4-methylphenol	109	18.0 ± 11.3		~100	~100				
4-Ethylphenol	122	2.7 ± 2.6		~100	~100		68 ± 11	26 ± 16	56 ± 14
Indole	117	0.54 ± 0.7		~100	~100		48 ± 12	17 ± 14	38 ± 11
Skatole	130	1.73 ± 1.8		98.9	98.9		69 ± 10	20 ± 16	62 ± 10
Reduced organic sulfur compounds	47						19 ± 21	16 ± 22	4 ± 12
Measurements by PTR-MS (n = 1)									
H_2S	35		102				–2	–21	15
Methanethiol	49		12.5				50	40	16
Dimethyl sulfide	63		12.2				29	18	13
Dimethyl disulfide	95	0.61 ± 0.1	1.0 ± 0.5	94	86	59			

[a] Determined by GC/MS (Kiel) or by MIMS (Randers).

were neutral, whereas the concentration of NO_3^- and NO_2^- had accumulated substantially in the Randers biofilter.

Elimination of odor compounds. The concentration of selected compounds and chemical groups in the air emissions from the pig stables and the capabilities in eliminating these compounds of the two SKOV-type biofilters investigated are presented in Table 1. Unbiased detection and quantification of DMDS is highly challenging for several reasons. As mentioned, on-line MS is not directly able to distinguish DMDS from phenol, since they have the same molecular weight. Using a DMDS mass fragment at *m/z* 79 does not solve this, since other compounds contribute to this mass (*p*-cresol in MIMS and benzene in PTR-MS). Analysis by GC/MS is associated with a bias due to dimerization/oxidation of methanethiol either during sampling or analysis (11, 29). Because methanethiol is most likely more abundant (24), this may lead to an overestimation of DMDS. Gas chromatographs with sulfur-specific detection, e.g., amperometric or chemiluminescence, suffer from high detection limits (ca. 3 to 10 ppb). PTR-MS detection of DMDS by means of *m/z* 97 by taking advantage of the ^{34}S-labeled isotope abundance, theoretically at 8.8% for a compound with two sulfur atoms, is biased by the presence of furfural, which would also contribute to *m/z* 97. In addition, the *m/z* 97 observed here was relatively close to the detection limit. As a compromise, DMDS was estimated by measuring the concentration of phenol by GC/MS analysis of Tenax-packed sorbent tubes and subtracting this from the sum of the concentrations of DMDS and phenol, $\Sigma_{DMDS+phenol}$, as estimated by PTR-MS. The ammonia emissions of ~10 and ~8 ppm were reduced to less than 0.02 and 0.01 ppm for the Randers and the Kiel filters, respectively. This removal was mainly accomplished by the first filter sections. The results are summarized in Table 1.

Activity of incubations. Incubations with unlabeled butyric acid and DMDS revealed removal from the headspace following first-order kinetics with rate constants of 0.48 h^{-1} and 0.02 h^{-1}, respectively, indicating the presence of active populations of butyric acid and DMDS-oxidizing bacteria. To incorporate sufficient activity of the ^{14}C-labeled substrates in the MAR microcosms, one [$^{14}C_2$]butyrate addition was adequate. The specific activity of [$^{14}C_2$]DMDS was relatively high (50 mCi $mmol^{-1}$), which necessitated a concentration of 0.1 mM DMDS in order not to lose the specified level of radioactivity (10 μCi).

DGGE profiling of fractionated DNA from SIP incubations. Diverse DGGE patterns were associated with the low-density gradient fractions, demonstrating a wide diversity of microorganisms present in the Kiel biofilter biofilm. For butyric acid SIP, one distinct ^{13}C-enriched band emerged in the heavy fractions after an incubation time of 7 and 45 h (data not shown) likely representing one highly active species. This ^{13}C-enriched band was accompanied by few other bands after 110 h

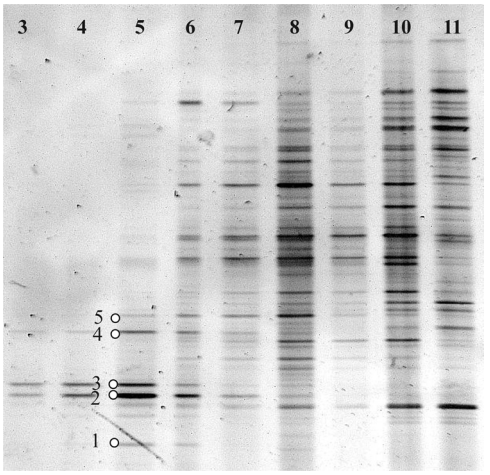

FIG. 2. DGGE fingerprints of SIP-incubated biofilter biofilm. During the 110 h of incubation, 3 mM [$^{13}C_4$]butyric acid was supplied, at depletion, as a substrate through 12 additions. PCR-amplified 16S rRNA genes from fractions 3 (heaviest) to 11 (lightest) are presented. The numbered bands were excised for DNA sequence analysis.

of incubation (Fig. 2) in the high-density fractions. Since a smear of fingerprints from the light fractions appeared in fraction 6, which was also observed in the unlabeled control (data not shown), fraction 5 (density, 1.732 g ml^{-1}) was chosen for further phylogenetic analysis. The DGGE bands retrieved from heavy DNA, fraction 5 (Fig. 2), yielded clear 16S rRNA gene sequences and were in agreement with clone library data, grouping with the *Actinobacteria* (Fig. 3).

No unique DGGE bands were apparent in the high-density fractions for the [$^{13}C_2$]DMDS incubations, suggesting insufficient ^{13}C labeling of DNA in microorganisms transforming the DMDS. Likely, adsorption to biofilm and particles trapped in the biofilm as observed by MAR analysis (see below) have overestimated the actual first-order consumption rate.

Butyric acid assimilators identified from SIP analysis. Phylogenetic trees were constructed from light DNA (fraction 10) and heavy DNA (fraction 5) using a total of 23 and 39 high-quality sequences, respectively. Twelve sequences from fraction 5 and three sequences of fraction 10 were of mitochondrial origin, most likely fungal, but their identity and exact role was not further resolved.

The most abundant phylum of the light DNA clone library was *Proteobacteria*, with all sequences affiliating with the beta subdivision (14 sequences; 61%), followed by *Bacteroidetes* (four sequences; 17%) and 1 sequence representing each of *Actinobacteria*, *Firmicutes*, *Verrucomicrobia*, candidate division OD1 and TM7 (4% each) (Fig. 3A). In the heavy DNA clone library, *Actinobacteria* was the most abundant phylum (31 sequences; 79%) (Fig. 3C), followed by *Betaproteobacteria* (3 sequences; 8%), *Gammaproteobacteria* (3 sequences; 8%), and *Alphaproteobacteria* (2 sequences; 5%) (Fig. 3B). At the genus level, only two genera (*Castellaniella* and *Microbacterium*) were shared among the low- and high-density DNA clone libraries. The community divergence between the fractions strongly indicated a shift in the microbial community caused by the incorporation of ^{13}C-labeled substrate.

A total of 13 operational taxonomic units (OTUs) were obtained from the ^{13}C-enriched DNA. Although the most abundant OTUs were closely related to the actinobacterial genera *Microbacterium*, *Rhodococcus* and *Propionibacterium*, sequences were also obtained affiliating *Gordonia*, *Dietzia* and *Janibacter* (*Actinobacteria*). The remaining seven OTUs contained one or two sequences each and affiliated with *Alpha-*, *Beta-*, and *Gammaproteobacteria*.

Substrate assimilation patterns from MAR-FISH analysis. MAR was conducted with both [$^{14}C_2$]butyric acid and [$^{14}C_2$]DMDS as substrates (Table 2), and precipitation of silver grains on top of individual cells distinguished MAR-positive cells from non-active members of the microbial community (Fig. 4). Radioactive substrate sorption to nonbiological objects in the biofilm was observed in pasteurized controls, but active substrate uptake was easily distinguished from sorption and precipitation, compared to the control.

Approximately 8% of the EUBmix-detectable community (all bacteria) took up [$^{14}C_2$]butyric acid, and the application of specific probes revealed that major assimilations were specifically carried out by two distinct morphological groups: rods and microcolony-forming cocci both targeted by the HGC69a probe (*Actinobacteria*), thereby confirming the SIP results (Fig. 4). A small fraction of *Comamonadaceae*-related bacteria also took up [$^{14}C_2$]butyric acid, although the MAR activity was low. DMDS was assimilated by ca. 20% of the EUBmix detectable community. *Actinobacteria* and *Bacteroidetes* (probe CF319ab) were identified as being part of the community taking up DMDS but, surprisingly, several ammonia-oxidizing bacteria (probe NSO190 in combination with BET42a, with typical nitrifier cell and microcolony morphology) were also labeled, although not found by SIP. Other spherical cells with an approximate diameter of 10 μm were also found to be MAR positive after [$^{14}C_2$]DMDS incubation but did not hybridize with the specific probes used here. Since eukaryotic cell structures were not conserved by fixation, these structures are hypothesized to represent fungal spores. However, these structures represented a minor part of the MAR-positive microbial community (<5%). Quantitative FISH analysis with the applied FISH probes did not reveal significant changes in the population due to isotope incubation (data not shown).

DISCUSSION

Biofiltration is a promising and cost-effective technology for the treatment of large volumes of industrial waste gas containing low concentrations of various VOCs. Butyrate- and reduced organic sulfur-containing compounds are commonly detected in emissions from pig facilities, but considerable elimination of the two compounds by biofiltration has been demonstrated (11). In the present study, we detected the removal efficiency of major groups of VOCs, as well as few single compounds, by two biofilters of the SKOV-type (Fig. 1). Upon high emission rates of carboxylic acids, removal efficiencies as high as 90% were observed, although the overall removal rate for one of the biofilters was slightly lower than previously reported for a SKOV-type filter (>75%) (11). This might be caused by an underestimation due to condensed water in the tubing during MIMS measurement. The removal of butyrate and other carboxylic acids was mainly attributed to the primary filters due to their hydrophilicity and high bioavailability, also

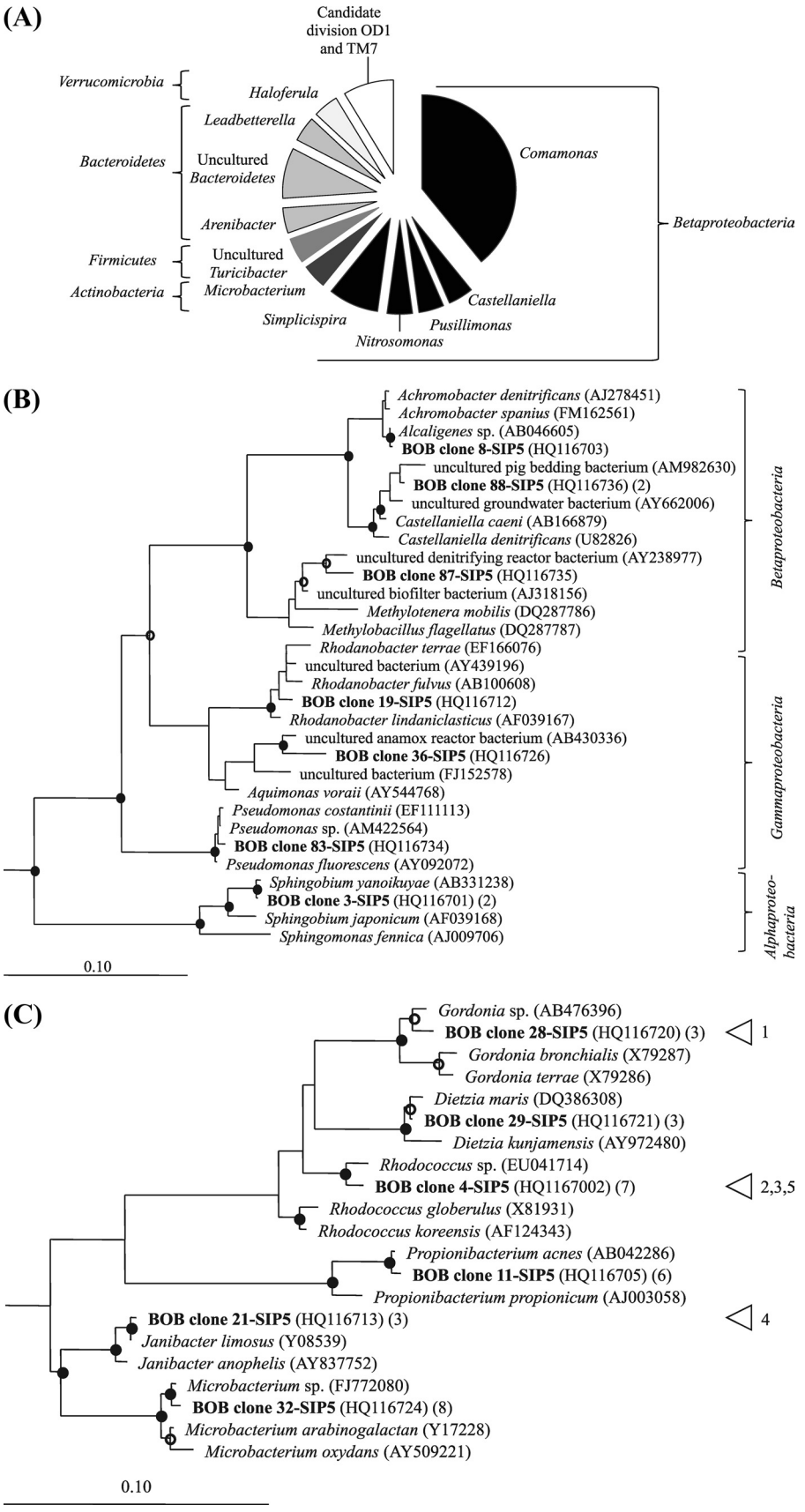
(A)
Candidate division OD1 and TM7
Verrucomicrobia
Haloferula
Leadbetterella
Bacteroidetes
Uncultured Bacteroidetes
Arenibacter
Firmicutes
Uncultured Turicibacter
Actinobacteria
Microbacterium
Simplicispira
Nitrosomonas
Pusillimonas
Castellaniella
Comamonas
Betaproteobacteria
(B)
Achromobacter denitrificans (AJ278451)
Achromobacter spanius (FM162561)
Alcaligenes sp. (AB046605)
BOB clone 8-SIP5 (HQ116703)
uncultured pig bedding bacterium (AM982630)
BOB clone 88-SIP5 (HQ116736) (2)
uncultured groundwater bacterium (AY662006)
Castellaniella caeni (AB166879)
Castellaniella denitrificans (U82826)
uncultured denitrifying reactor bacterium (AY238977)
BOB clone 87-SIP5 (HQ116735)
uncultured biofilter bacterium (AJ318156)
Methylotenera mobilis (DQ287786)
Methylobacillus flagellatus (DQ287787)
Rhodanobacter terrae (EF166076)
uncultured bacterium (AY439196)
Rhodanobacter fulvus (AB100608)
BOB clone 19-SIP5 (HQ116712)
Rhodanobacter lindaniclasticus (AF039167)
uncultured anamox reactor bacterium (AB430336)
BOB clone 36-SIP5 (HQ116726)
uncultured bacterium (FJ152578)
Aquimonas voraii (AY544768)
Pseudomonas costantinii (EF111113)
Pseudomonas sp. (AM422564)
BOB clone 83-SIP5 (HQ116734)
Pseudomonas fluorescens (AY092072)
Sphingobium yanoikuyae (AB331238)
BOB clone 3-SIP5 (HQ116701) (2)
Sphingobium japonicum (AF039168)
Sphingomonas fennica (AJ009706)
Betaproteobacteria
Gammaproteobacteria
Alphaproteo-bacteria
0.10
(C)
Gordonia sp. (AB476396)
BOB clone 28-SIP5 (HQ116720) (3)
Gordonia bronchialis (X79287)
Gordonia terrae (X79286)
Dietzia maris (DQ386308)
BOB clone 29-SIP5 (HQ116721) (3)
Dietzia kunjamensis (AY972480)
Rhodococcus sp. (EU041714)
BOB clone 4-SIP5 (HQ1167002) (7)
Rhodococcus globerulus (X81931)
Rhodococcus koreensis (AF124343)
Propionibacterium acnes (AB042286)
BOB clone 11-SIP5 (HQ116705) (6)
Propionibacterium propionicum (AJ003058)
BOB clone 21-SIP5 (HQ116713) (3)
Janibacter limosus (Y08539)
Janibacter anophelis (AY837752)
Microbacterium sp. (FJ772080)
BOB clone 32-SIP5 (HQ116724) (8)
Microbacterium arabinogalactan (Y17228)
Microbacterium oxydans (AY509221)
1
2,3,5
4
0.10

TABLE 2. Uptake of butyric acid and DMDS as determined by MAR and identified by FISH

Organism	FISH counts (% of EUBmix)	Substrate (% MAR positive)[a]	
		Butyric acid	DMDS
Bacteria (EUBmix)		8	20
Comamonadaceae (BONE)	12	1 (+)	–
Other *Betaproteobacteria* (BTWO)	12	–	–
Betaproteobacterial AOBs (NSO190)	15	–	10 (++)
Gammaproteobacteria (GAM42a)	3	–	–
Sphingobacteria and *Flavobacteria* (CF319a)	7	–	6 (+)
Actinobacteria (HGC69a)	30	22 (++)	20 (+)

[a] –, No uptake; +, weak but positive uptake; ++, strong uptake.

in accordance with previous observations (11). High removal efficiencies were generally obtained for aromatic compounds (48 to 89%), which in all cases mainly were accomplished by the secondary filter. Khammar et al. (23) found a similar stratification, where oxygenated compounds were removed at the inlet of a lab-scale biofilter, while aromatics were reduced deeper inside the biofilter, reflecting the hydrophobicity and bioavailable character of these compounds. The abundances of reduced sulfur compounds, as measured by PTR-MS, were close to previously reported values for emissions from pig production (24). Their elimination in the biofilter mainly occurred in the primary filter, but was relatively weak, possibly due to low mass transfer of these volatile hydrophobic compounds. Finally, ammonia was completely eliminated by the primary filter. Ammonia was likely oxidized by nitrifying bacteria, which have been detected in a similar biofilter (20), causing the accumulated concentrations of nitrate and nitrite. Thus, various VOCs with different properties and ammonia emitted from the pig facility were removed by the SKOV-type biofilter.

A combination of the molecular tools DNA-SIP and MAR-FISH was used to isotopically label and identify microorganisms assimilating butyric acid and DMDS in a SKOV-type biofilter located near Kiel, Germany. DNA-SIP identifies bacteria that assimilate a specific substrate by purifying entire genomes of the active organisms, but a longer incubation time is required; the active cells must undergo at least two to three cell divisions. On the other hand, MAR-FISH quickly detects all incorporated radiolabeled macromolecules without the requirement of cell division and yields quantitative information on the relative abundance of the active community. However, MAR-FISH is limited to the populations targeted by the selected probes. In general, obtaining sufficient isotopic labeling of the active portion of the microbial community is complicated by efforts to minimize incubation times and maintain substrate concentrations as close to *in situ* environmental conditions as possible (35, 38). The compromise used in the present study was to apply concentrations of butyrate in the SIP and MAR microcosms ~1 order of magnitude higher than those measured in the exhaust air, whereas the DMDS concentration was 4 and 5 orders of magnitude higher in the SIP and MAR microcosms than those measured, respectively. The elevated concentrations and long incubation time applied to the DMDS microcosms might have biased the results from the SIP experiment. However, no changes in the abundance of any of the investigated FISH populations were detected as a consequence of the incubations. Despite the limitation of each method and a relatively low number of sequences analyzed, initial information toward characterizing active and uncultured microorganisms involved in DMDS and butyrate uptake was obtained.

The 16S rRNA gene sequences derived from the [$^{13}C_4$] butyric acid microcosm heavy DNA demonstrated that *Actinobacteria* were enriched, including the genera *Microbacterium*, *Rhodococcus*, and *Propionibacterium*, followed by *Dietzia*, *Gordonia*, and *Janibacter*. In addition, several OTUs within *Alpha-*, *Beta-*, and *Gammaproteobacteria* were detected. MAR-FISH confirmed *Actinobacteria* as the dominating butyric acid-consuming phylum. Several BONE23a (*Comamonadaceae*) positive cells were also associated with butyrate uptake according to MAR-FISH. However, no SIP-identified *Betaproteobacteria* were identified by MAR-FISH, and none of the clones from the labeled DNA contained the target site for the probe BONE23a. Apart from the direct use of butyric acid as an energy and carbon source, the compound has also been reported to be assimilated in storage compounds such as poly-β-hydroxybutyrate granules in different microorganisms, which could possibly explain this result (28). In general, *Actinobacteria* are often found to be a quantitatively important part of the microbial community in biofilters treating VOC (15) and were also found to constitute up to 16% of the total community in a similar air filter treating exhaust air from a pig farm (26). The aerobic *Gordonia* and *Rhodococcus* have previously been detected by cultivation in biofilter communities (23), and several other genera of *Actinobacteria* such as *Dietzia*, *Gordonia*, *Janibacter*, and *Rhodococcus* are known to oxidize butyric acid as detected in pure culture studies (4, 7), but the ability has not yet been shown with *in situ* experiments. Furthermore, *Gordonia* has previously been associated with the degradation of hexane using stable isotope-based phospholipid fatty acid analysis on a biofilter treating air from an oil mill (13), which could suggest a more general role for species of this genus. The observation of *Propionibacterium* in large numbers in the [$^{13}C_4$]butyric acid clone library was somewhat surprising be-

FIG. 3. Phylogenetic affiliations of obtained 16S rRNA sequences from the DNA-SIP experiment. (A) Microbial community composition of sequences obtained from the light DNA fraction (10) at the genus level, with divisions indicated with curly brackets. (B and C) Maximum-likelihood tree of *Proteobacteria*-related (B) and *Actinobacteria*-related (C) sequences obtained from the heavy fraction (5). The closest GenBank uncultured sequences and reference strains are shown, and the accession numbers and number of sequences within each OTU which is represented by only one sequences is indicated in parentheses). Bootstrap support is indicated by open circles (○) for values ≥75% and filled circles (●) for values ≥95%. The trees were rooted using *Campylobacter insulaenigrae* (AJ620504) and *C. lari* (L04316) (B) and *Patulibacter americanus* (AJ871306) and *P. minatonensis* (AB193261) (C). The approximate phylogenetic affiliation of the excised DGGE sequences from Fig. 2 is indicated by symbols along the right side of the figure.

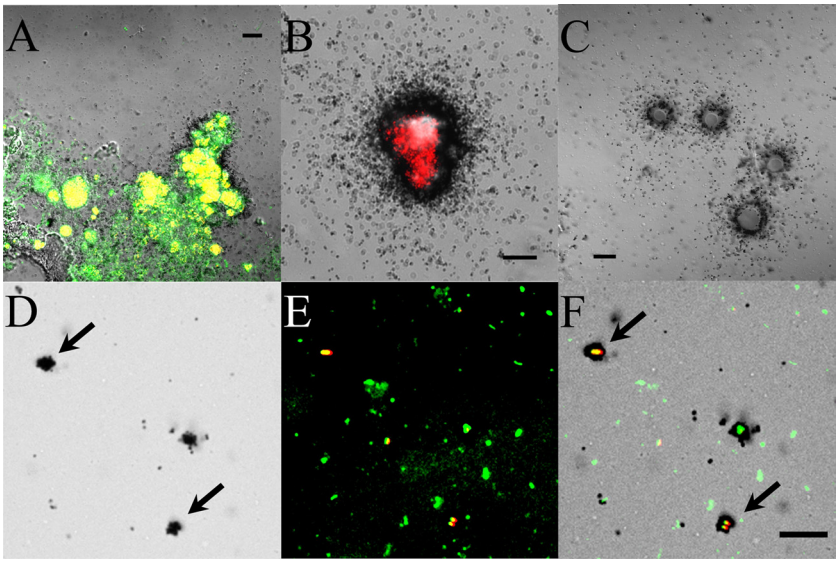

FIG. 4. Microautoradiography (MAR) combined with fluorescence *in situ* hybridization (FISH) performed on biofilter biofilm. The images show representative MAR-positive microcolonies and single cells after 19 h of incubation of the biofilm with 0.1 mM [$^{14}C_2$]DMDS (A to C) and 260 h with 3 mM [$^{14}C_2$]butyric acid (D to F). The images are composed of a fluorescence micrograph after hybridization with the probes NSO190 (A and B) specific for ammonia oxidizing *Betaproteobacteria* and EUBmix targeting all bacteria (A). Cells appearing yellow hybridized with both probes (A). Panel C shows an example of single cells with fungal morphology which had assimilated [$^{14}C_2$]DMDS. Panel E shows cells hybridized with a Cy3-labeled probe (red) specific for *Actinobacteria* (HGC69a) and EUBmix (green), and panel F is the superimposed image from panels D and E. Cells MAR positive and targeted by the HGC69a probes are indicated by arrows. Scale bars, 10 μm.

cause species of this genus are considered to be primary fermenting bacteria producing propionic acid (48). However, the existence of anoxic or even microaerophilic pockets in the biofilm may create a habitat for fermentation of butyric acid by *Propionibacterium* or an oxic metabolic pathway may exist. The finding of *Gammaproteobacteria* in the [$^{13}C_4$]butyric acid clone library was not confirmed by MAR-FISH and could thus implicate the presence of cross-feeding, [^{12}C]DNA contamination, cells with low activity, ribosomal content, or substrate incorporation, or a group of *Gammaproteobacteria* without the GAM42a-target site. Various types of *Gammaproteobacteria* have been proposed to be important degraders of butyric acid in a biofilter by means of cultivation, but Sheridan et al. (45) also noted that *Gammaproteobacteria* are fast growing in cultures, possibly overgrowing important microorganisms. Major parts of the biofilm in the SKOV-type biofilter are oxic (20), which reflects the aerobic microbial community taking up butyric acid identified in the present study with the exception of *Propionibacterium*.

Actinobacteria were also the main community members affiliated with the oxidation and assimilation of DMDS carbon, followed by the betaproteobacterial ammonia-oxidizing bacteria (AOB) (NSO190) and the group of *Sphingobacteria* and *Flavobacteria* (*Bacteroidetes*; CF319ab). AOB assimilation of DMDS was unexpected because AOB are normally considered autotrophic and also because of the addition of thiourea to the incubations. Thiourea specifically inhibits the enzyme ammonia monooxygenase (19), so our results suggest that the DMDS was taken up by an alternative metabolism. The finding of AOB assimilating DMDS carbon could also imply cross-feeding. Long incubation times of the [$^{14}C_4$]DMDS microcosm may have resulted in the production of [^{14}C]CO_2 from the DMDS-oxidizing bacteria, which was assimilated by the autotrophic AOB. Although the MAR signals were the most dominant in the systems, no AOB sequences were identified in the SIP experiment. However, the uptake of small amount of organic substrates such as *p*-cresol, pyruvate, and triclosan by nitrifiers has previously been described (10, 21, 41) and, from whole-genome sequencing of *Nitrosomonas europaea*, genes encoding enzymes for catabolic oxidation have been identified (6). No associations between AOB microcolonies and other microorganisms were observed by FISH using any of the probe combinations. The exact role of AOB in DMDS transformation therefore remains unknown, although our results show that they have a metabolic role to play in these biofilter environments. Several examples have shown a complete oxidation of DMDS to sulfate (46), but since *Actinobacteria*, *Flavobacteria*, and *Sphingobacteria* are not typically involved in the sulfur cycle, the biochemical pathways remain unknown for these species. The biodegradation kinetics of DMDS in the biofilter needs further investigation in order to understand the flow of elements from DMDS.

In conclusion, the two isotope-based molecular methods used here provided complementary data, which revealed the active odor-eliminating microbial communities in these biofilter environments. The results underline a strong contribution of the *Actinobacteria* in the uptake of both butyric acid and DMDS in the biofilter, although more specific probes and targeted cultivation and metagenomic efforts would help us to better understand the precise metabolic roles of each of the detected genera in these systems. The findings show, for the first time, active microbial consortia that metabolize specific compounds in the exhaust air from pig facilities. Identifying the roles of specific organisms within air biofilter communities

represents an important first step toward understanding the stability and efficiency of such biofilters, optimizing the removal of key compounds, and guiding targeted cultivation and genomic efforts that might result in the development of designer consortia for industrial bioaugmentation applications.

ACKNOWLEDGMENTS

This study was supported by the Danish Ministry of Food, Agriculture, and Fisheries as part of the program Function of Biological Airfilters to J.L.N. J.D.N. acknowledges funding of a Discovery Grant from the Natural Science and Engineering Research Council of Canada.

We thank Lise Bonne Guldberg from SKOV A/S for cooperation and kindly providing the biofilm sample and information about the biofilter near Kiel, Germany, and to farm owner Torben Høj Lauersen for assistance with equipment and information about his farm near Randers, Denmark. We also acknowledge Michael Jørgen Hansen, Aarhus University, for providing GC/MS data.

REFERENCES

1. **Alexandrino, M., C. Knief, and A. Lipski.** 2001. Stable-isotope-based labeling of styrene-degrading microorganisms in biofilters. Appl. Environ. Microbiol. **67:**4796–4804.
2. **Amann, R., J. Snaidr, M. Wagner, W. Ludwig, and K. H. Schleifer.** 1996. *In situ* visualization of high genetic diversity in a natural microbial community. J. Bacteriol. **178:**3496–3500.
3. **Bartram, A. K., C. Poon, and J. D. Neufeld.** 2009. Nucleic acid contamination of glycogen used in nucleic acid precipitation and assessment of linear polyacrylamide as an alternative co-precipitant. Biotechniques **47:**1016–1019.
4. **Bodtker, G., I. V. Hvidsten, T. Barth, and T. Torsvik.** 2009. Hydrocarbon degradation by *Dietzia* sp. A14101 isolated from an oil reservoir model column. Antonie Van Leeuwenhoek **96:**459–469.
5. **Butler, J. A. V., and C. N. Ramchandani.** 1935. The solubility of nonelectrolytes. II. The influence of the polar group on the free energy of hydration of aliphatic compounds. J. Chem. Soc. **1935:**952–955.
6. **Chain, P., et al.** 2003. Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europaea*. J. Bacteriol. **185:**2759–2773.
7. **Chatterjee, S., S. Mallick, and T. K. Dutta.** 2005. Pathways in the degradation of hydrolyzed alcohols of butyl benzyl phthalate in metabolically diverse *Gordonia* sp. strain MTCC 4818. J. Mol. Microbiol. Biotechnol. **9:**110–120.
8. **Chen, L., and S. J. Hoff.** 2009. Mitigating odors from agricultural facilities: a review of literature concerning biofilters. Appl. Eng. Agric. **25:**751–766.
9. **Daims, H., A. Bruhl, R. Amann, K. H. Schleifer, and M. Wagner.** 1999. The domain-specific probe EUB338 is insufficient for the detection of all *Bacteria*: development and evaluation of a more comprehensive probe set. Syst. Appl. Microbiol. **22:**434–444.
10. **Daims, H., et al.** 2001. Nitrification in sequencing biofilm batch reactors: lessons from molecular approaches. Water Sci. Technol. **43:**9–18.
11. **Feilberg, A., A. P. S. Adamsen, S. Lindholst, M. Lyngbye, and A. Schafer.** 2010. Evaluation of biological air filters for livestock ventilation air by membrane inlet mass spectrometry. J. Environ. Qual. **39:**1085–1096.
12. **Fox, J. G., et al.** 1995. *Helicobacter bilis* sp. nov., a novel *Helicobacter* species isolated from bile, livers, and intestines of aged, inbred mice. J. Clin. Microbiol. **33:**445–454.
13. **Friedrich, M. M., and A. Lipski.** 2010. Characterisation of hexane-degrading microorganisms in a biofilter by stable isotope-based fatty acid analysis, FISH and cultivation. Appl. Microbiol. Biotechnol. **85:**1189–1199.
14. **Friedrich, U., K. Prior, K. Altendorf, and A. Lipski.** 2002. High bacterial diversity of a waste gas-degrading community in an industrial biofilter as shown by a 16S rDNA clone library. Environ. Microbiol. **4:**721–734.
15. **Friedrich, U., H. Van Langenhove, K. Altendorf, and A. Lipski.** 2003. Microbial community and physicochemical analysis of an industrial waste gas biofilter and design of 16S rRNA-targeting oligonucleotide probes. Environ. Microbiol. **5:**183–201.
16. **Green, S. J., M. B. Leigh, and J. D. Neufeld.** 2010. Denaturing gradient gel electrophoresis (DGGE) for microbial community analysis, p. 4137–4158. *In* K. Timmis (ed.), Hydrocarbon microbiology. Springer, Berlin, Germany.
17. **Hort, C., S. Gracy, V. Platel, and L. Moynault.** 2009. Evaluation of sewage sludge and yard waste compost as a biofilter media for the removal of ammonia and volatile organic sulfur compounds (VOSCs). Chem. Engl. J. **152:**44–53.
18. **Huber, T., G. Faulkner, and P. Hugenholtz.** 2004. Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics **20:**2317–2319.
19. **Hyman, M. R., C. Y. Kim, and D. J. Arp.** 1990. Inhibition of ammonia monooxygenase in *Nitrosomonas europaea* by carbon-disulfide. J. Bacteriol. **172:**4775–4782.
20. **Juhler, S., et al.** 2009. Distribution and rate of microbial processes in an ammonia-loaded air filter biofilm. Appl. Environ. Microbiol. **75:**3705–3713.
21. **Keener, W. K., and D. J. Arp.** 1994. Transformations of aromatic compounds by *Nitrosomonas europaea*. Appl. Environ. Microbiol. **60:**1914–1920.
22. **Keeney, D. R., and D. W. Nelson.** 1982. Nitrogen: inorganic forms, p. 643–698. *In* A. L. Page et al. (ed.), Method for soil analysis. 2. Chemical and microbiological properties, 2nd ed. Agronomy monograph 9. ASA and SSSA, Madison, WI.
23. **Khammar, N., et al.** 2005. Link between spatial structure of microbial communities and degradation of a complex mixture of volatile organic compounds in peat biofilters. J. Appl. Microbiol. **98:**476–490.
24. **Kim, K. Y., et al.** 2007. Sulfuric odorous compounds emitted from pig-feeding operations. Atmos. Environ. **41:**4811–4818.
25. **Knief, C., K. Altendorf, and A. Lipski.** 2003. Linking autotrophic activity in environmental samples with specific bacterial taxa by detection of ^{13}C-labeled fatty acids. Environ. Microbiol. **5:**1155–1167.
26. **Kristiansen, A., et al.** 2011. Bacterial community structure of a full-scale biofilter treating pig house exhaust air. Syst. Appl. Microbiol. **34:**344–352.
27. **Lane, D. J.** 1991. 16S/23S rRNA sequencing, p. 115–175. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Chichester, United Kingdom.
28. **Lemos, P. C., L. S. Serafim, and M. A. M. Reis.** 2006. Synthesis of polyhydroxyalkanoates from different short-chain fatty acids by mixed cultures submitted to aerobic dynamic feeding. J. Biotechnol. **122:**226–238.
29. **Lestremau, F., F. A. T. Andersson, and V. Desauziers.** 2004. Investigation of artifact formation during analysis of volatile sulphur compounds using solid phase microextraction (SPME). Chromatographia **59:**607–613.
30. **Ludwig, W., et al.** 2004. ARB: a software environment for sequence data. Nucleic Acids Res. **32:**1363–1371.
31. **Manz, W., R. Amann, W. Ludwig, M. Vancanneyt, and K. H. Schleifer.** 1996. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum *Cytophaga-Flavobacter-Bacteroides* in the natural environment. Microbiology **142:**1097–1106.
32. **Manz, W., R. Amann, W. Ludwig, M. Wagner, and K. H. Schleifer.** 1992. Phylogenetic oligodeoxynucleotide probes for the major subclasses of *Proteobacteria*: problems and solutions. Syst. Appl. Microbiol. **15:**593–600.
33. **Mobarry, B. K., M. Wagner, V. Urbain, B. E. Rittmann, and D. A. Stahl.** 1996. Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. Appl. Environ. Microbiol. **62:**2156–2162.
34. **Muyzer, G., E. C. Dewaal, and A. G. Uitterlinden.** 1993. Profiling of complex microbial-populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes-coding for 16S ribosomal-RNA. Appl. Environ. Microbiol. **59:**695–700.
35. **Neufeld, J. D., M. G. Dumont, J. Vohra, and J. C. Murrell.** 2007. Methodological considerations for the use of stable isotope probing in microbial ecology. Microb. Ecol. **53:**435–442.
36. **Neufeld, J. D., et al.** 2007. DNA stable-isotope probing. Nat. Protoc. **2:**860–866.
37. **Nielsen, J. L.** 2009. Protocol for fluorescence *in situ* hybridization (FISH) with rRNA-targeted oligonucleotides, p. 73–84. *In* P. H. Nielsen, H. Daims, and H. Lemmer (ed.), FISH handbook for biological wastewater treatment. IWA Publishing, London, England.
38. **Nielsen, J. L., and P. H. Nielsen.** 2005. Advances in microscopy: microautoradiography of single cells, p. 237–256. *In* J. R. Leadbetter (ed.), Environmental microbiology, vol. 397. Elsevier Academic Press, Inc., San Diego, CA.
39. **Pruesse, E., et al.** 2007. SILVA: a comprehensive online resource for quality checked and aligned rRNA sequence data compatible with ARB. Nucleic Acids Res. **35:**7188–7196.
40. **Revar, S., and J. M. Morgan-Sagastume.** 2005. Methods of odor and VOC control, p. 29–63. *In* Z. Shareefdeen and A. Singh (ed.), Biotechnology for odor and air pollution control. Springer, Berlin, Germany.
41. **Roh, H., et al.** 2009. Biodegradation potential of wastewater micropollutants by ammonia-oxidizing bacteria. Chemosphere **77:**1084–1089.
42. **Roller, C., M. Wagner, R. Amann, W. Ludwig, and K. H. Schleifer.** 1994. *In situ* probing of gram-positive bacteria with high DNA G+C content using 23S rRNA-targeted oligonucleotides. Microbiology **140:**2849–2858.
43. **Schiffman, S. S., J. L. Bennett, and J. H. Raymer.** 2001. Quantification of odors and odorants from swine operations in North Carolina. Agric. For. Meteorol. **108:**213–240.
44. **Sheridan, B., T. Curran, V. Dodd, and J. Colligan.** 2002. Biofiltration of odour and ammonia from a pig unit: a pilot-scale study. Biosyst. Eng. **82:**441–453.
45. **Sheridan, B. A., T. P. Curran, and V. A. Dodd.** 2003. Biofiltration of n-butyric acid for the control of odour. Bioresour. Technol. **89:**199–205.
46. **Sipma, J., et al.** 2004. Potentials of biological oxidation processes for the treatment of spent sulfidic caustics containing thiols. Water Res. **38:**4331–4340.
47. **Smith, V. H.** 2003. Eutrophication of freshwater and coastal marine ecosystems: a global problem. Environ. Sci. Pollut. Res. **10:**126–139.
48. **Stackebrandt, E., C. S. Cummins, and J. L. Johnson.** 2007. Family *Propionibacteriaceae*: the genus *Propionibacterium*, p. 400–418. *In* M. Dworkin,

S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt (ed.), The prokaryotes, vol. 3, 3rd ed. Springer, New York, NY.
49. **Stahl, D. A., and R. Amann.** 1991. Development and application of nucleic acid probes, p. 205–248. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Ltd., Chichester, England.
50. **Trabue, S. L., K. D. Scoggin, H. Li, R. Burns, and H. Xin.** 2008. Field sampling method for quantifying odorants in humid environments. Environ. Sci. Technol. **42:**3745–3750.
51. **Vitenberg, A. G., B. V. Ioffe, Z. S. Dimitrova, and I. L. Butaeva.** 1975. Determination of gas-liquid partition-coefficients by means of gas-chromatographic analysis. J. Chromatogr. **112:**319–327.
52. **von Keitz, V., A. Schramm, K. Altendorf, and A. Lipski.** 1999. Characterization of microbial communities of biofilters by phospholipid fatty acid analysis and rRNA targeted oligonucleotide probes. Syst. Appl. Microbiol. **22:**626–634.
53. **Wallner, G., R. Amann, and W. Beisker.** 1993. Optimizing fluorescent *in situ* hybridization with ribosomal-RNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. Cytometry **14:**136–143.